Chapter 10

Hydrogels by Stereocomplex Formation and Their Use as Drug Delivery Matrices

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Block and graft copolymer hydrogels, physically crosslinked through stereocomplex formation of poly- or oligo(lactic acid) chains of opposite chirality, are described. Protein-loaded hydrogels of dextran-*g*-oligo(L/D-lactic acid) are obtained from aqueous solutions of the two polymers (containing L- and D-grafts, respectively). These hydrogels degraded in a period of 1-7 days, depending on polymer composition. Lysozyme was quantitatively released with full preservation of its activity.

Introduction

Hydrogels are polymeric networks, which absorb and retain large amounts of water. In general, hydrogels possess a good biocompatibility. Their hydrophilic surface has a low interfacial free energy in contact with body fluids, which results in a low tendency for proteins and cells to adhere to these surfaces. Moreover, the soft and rubbery nature of hydrogels minimizes irritation to surrounding tissue. Therefore, hydrogels have found widespread application in different technological areas, e.g. as materials for contact lenses and protein separation, matrices for cell-encapsulation and devices for the controlled release of drugs and proteins.(*1–5*)

For many applications, such as drug delivery, it is advantageous that the hydrogels are biodegradable. Labile bonds can be present either in the polymer used to prepare the gel or in the crosslinks. These bonds can be broken under physiological conditions, in most of the cases by hydrolysis, either enzymatically or chemically.(*5*) It is of great interest to have control over the degradation kinetics; in other words, to have control over the parameters by which the degradation characteristics can be tailored. Moreover, once the hydrogels are implanted it is important that the formed degradation products have a low toxicity meaning that the formed compounds can either be metabolized into harmless products or can be excreted by the renal filtration process. The nature of the formed degradation products can be tailored by a rational and proper selection of the hydrogel building blocks.

Both chemical and physical methods have been used to create hydrogels.(6) In chemically crosslinked gels, covalent bonds are present between different polymer chains. In physically crosslinked gels, dissolution is prevented by physical interactions, which exist between different polymer chains. In recent years, there is an increasing interest in physically crosslinked gels, especially in which the gel formation occurs under mild conditions in the absence of organic solvents. The main reason is that the use of crosslinking agents and organic solvents to prepare such hydrogels is avoided. These agents and solvents can not only affect the integrity of the substances to be entrapped (e.g. proteins, cells), but they are often toxic compounds which have to be removed/extracted from the gels before they can be applied. To create physically crosslinked gels a great variety of methods have been applied, including ionic, hydrophobic and hydrogen bond interactions.(6) Also the formation of crystalline domains is a tool to create physical crosslinks. The latter includes the formation degradable stereocomplexes, which is the subject of this contribution. In this context, we will describe our newly developed biodegradable hydrogel system based on biocompatible substances, *i.e.* dextran and lactic acid oligomers. These hydrogels can be prepared from pure aqueous solutions and can entrap and release proteins and enzymes without affecting their integrity.

Macromolecular Stereocomplexes

In low molecular weight compounds possessing a chiral center, the formation of racemic crystals upon mixing the two enantiomers is a well-known phenomenon. A higher melting temperature (T_m) is frequently observed for the racemic crystallites than for each of the enantiomers. For example, D- and L-tartaric acid have a T_m of 173 °C, whereas for its racemic mixture a T_m of 206 °C is detected.(*7*)

In polymers of opposite chirality the formation of racemic crystallites has also been observed. In the literature, the formation of such racemic crystallites has been referred to as stereocomplexes and was first described by Dumas *et al.* in 1972.(*8*) They reported a melting point of 165 °C for an optically active poly(*t*-butyl-thiirane) as compared to 205 °C for the corresponding blend of poly(*R*-*t*-butyl-thiirane) and poly(*S*-*t*-butyl-thiirane). The difference in melting temperature was due to different crystal structures, as reported by Matsubayashi *et al.*.(*9*) Stereocomplexes were also observed for mixtures of the R- and S-forms of poly(α-methylbenzyl methacrylate),(*10*) poly(α-methyl-α-ethyl-β-propiolactone) (PMEPL),(*11*) poly(γ-benzyl glutamate),(12) poly(β-benzyl aspartate),(*13*) and α-olefin-carbon monoxide poly(1,4-ketone)s.(*14*)

It should be noted that the term stereocomplex is not exclusively used for racemic crystallites formed by chemically identical polymers of opposite chirality. Earlier, this term was used to describe the interaction between syndiotactic and isotactic polymers.(*15, 16*) Since these polymers do not have the same chemical structure, the term stereo-selective complexes, as suggested by Lohmeyer *et al.* is to be preferred in these cases rather than the term stereocomplexes.(*17*)

PLA Stereocomplex

Poly(lactic acid) (PLA) is a polyester, which consists of repeating units of lactic acid. Lactic acid, 2-hydroxypropionic acid, contains a chiral center and can therefore be in the L- or D-configuration. PLA is usually obtained by ring-opening polymerization of lactide, the cyclic dimer of lactic acid. Bulk polymerization of lactide with retention of stereochemistry can be carried out in the melt at 130 °C in the presence of the catalyst tin octoate.(*18, 19*) PLLA and PDLA, the homopolymers of L-lactic acid and D-lactic acid respectively, are semicrystalline materials. High molecular weight PLA, of either stereoisomer, has a melting temperature (T_m) of 170 °C, a melting enthalpy (ΔH_m) of 70 J/g, and a glass transition temperature (T_g) of 60 °C.(*20*) In blends of high molecular weight PDLA and PLLA, a phase with a higher T_m (230 °C) is observed. This phase is ascribed to stereocomplex formation. Racemic crystals were also

observed for the monomer lactide: the melting point (T_m) of D,D-lactide or L,L lactide is 95 °C, whereas the 1:1 racemate of D,D-lactide and L,L-lactide has a higher melting point of 126 °C.(*21*) The ability of PLA to form stereocomplexe was first described in a patent publication by Murdoch and Loomis,(*22*) and th first paper on these complexes was published by Ikada *et al.*(*23*)

By X-ray structure analysis, the unit cell of the crystals in the homopolymer of lactic acid was found to contain two 10_3 helices; poly(L-lactide) consists o left-handed helical chains and poly(D-lactide) of right-handed helical chains.(*24* In the stereocomplex crystal, a poly(L-lactide) segment and a poly(D-lactide segment are packed side by side in a 1:1 ratio of L and D monomer units an packed laterally in parallel fashion.(*24*) The unit cell of the complex contain three L- and three D-monomer units of the PLLA and PDLA helices, which ca be packed more densely than left-handed or right-handed helices alone. Eac PLLA and PDLA forms a more compact 3_1 helix in the complex crystal.(*24–26* The following mechanism of growth for the triangular lamellar stereocomple crystal was suggested by Brizzolara *et al.*:(*27*) As crystallization starts, fo example, one PDLA helix will be surrounded by three PLLA helices. Because c the triangular shape of the 3_1 helix, a triangular nucleus is thus formed whos respective sides are built up exclusively by PLLA. In the next step a PDLA laye grows on the crystal structure and then again a PLLA layer grows onto th PDLA layer and so on. Van der Waals forces between the helices cause specific energetic interaction-driven packing of the helices. These interactio cause the higher stability and consequently the higher melting point of th stereocomplex.(*27*)

PLA stereocomplexes were studied extensively as a new class biodegradable materials with higher mechanical strength, improved therm stability, and less sensitivity to hydrolysis than synthetic polyesters such a poly(glycolic acid) and PLA.(*28*) Stereocomplex PLA fibers were prepared b spinning from a mixed solution of PDLA and PLLA, and by dry spinning from melt of PDLA and PLLA to obtain reinforced materials, which are stronger tha PLLA.(*29*) However, PLA stereocomplexes have some drawbacks. They a highly resistant to degradation and thereby adversely affect th biocompatibility.(*30–33*) The poor biodegradation and biocompatibility of th PLA stereocomplexes resulted in a decreased interest in these system Nevertheless, at present stereocomplexes have regained interest for applicatio in drug delivery systems.

Hydrogels Based on Stereocomplex Formation

Stereocomplex formation between PLLA and PDLA, as described abov has been applied by several groups for the preparation of biodegradab

hydrogels. The general feature of these hydrogels is that polymers or oligomers of either L-lactic acid or D-lactic acid are attached to a water-soluble polymer in the form of block or graft copolymers. Association takes place in crystalline domains (stereocomplexes) upon mixing the two polymers (one containing L-lactic acid, the other containing D-lactic acid), providing the physical crosslinks. We would like to classify PLA stereocomplex hydrogels as follows:

1. Hydrogels containing high molecular weight (HMW) PLA chains. The individual enantiomeric polymers (PLLA and PDLA) are already crystalline and mostly insoluble in water. Mixing should therefore take place from organic solutions or in the melt, and the resulting blend can subsequently be swollen in contact with water. The difference of these stereocomplex hydrogels with respect to hydrogels containing PLLA or PDLA alone is that the crystalline domains are more stable and resistant to hydrolytic degradation.
2. Hydrogels containing oligomeric lactic acid (OLA) chains, whose individual enantiomeric polymers (OLLA and ODLA) can be soluble in water when the lactic acid content is sufficiently low. This provides the unique opportunity to form hydrogels by stereocomplex crystallization from aqueous solutions.

Stereocomplex Hydrogels Containing HMW PLA.

Stereocomplex formation between triblock copolymers of PLLA-PEG-PLLA and PDLA-PEG-PDLA (PEG = poly(ethylene glycol)) was studied with the aim to prepare hydrogels.(*34, 35*) The release of bovine serum albumin (BSA) from microspheres based on these triblock copolymers, has been studied by Lim *et al.* and compared with the release of BSA from microspheres prepared with one enantiomeric form of the triblock copolymer and with PLA microspheres.(*34*) The protein-loaded microspheres were prepared by a double-emulsion solvent evaporation method. The stereocomplex and single enantiomeric triblock copolymer microspheres showed a slightly larger burst release than PLA microspheres, which is likely caused by the higher water-uptake capacity of the PEG-containing microspheres. Although the morphology of the stereocomplex microspheres was clearly deviating, the release of BSA was similar to the single enantiomeric triblock copolymer microspheres.

Stereocomplex Hydrogels Containing OLA.

The triblock copolymers mentioned in the previous section are water-soluble when the hydrophobic PLA blocks are sufficiently short. The maximum

length of the lactic acid blocks for rendering water-solubility decreases with decreasing length of the PEG blocks used. For example, PEG-block with a molecular weight of 13.000 g mol^{-1} substituted with on average 26 lactic acid repeating units on each side of the PEG block are water-soluble and can form stereocomplexes upon mixing of aqueous solutions of each enantiomer.(*35*)

Recently, another system has been prepared by Lim *et al.*, based on stereocomplex formation by enantiomeric oligo(lactic acid) (OLA) side chains grafted onto poly(2-hydroxyethyl methacrylate) (PHEMA) (poly(HEMA-*g*-oligolactate)s).(*36*) The system was prepared by casting a film from poly(HEMA-*g*-oligo(L)lactate) and poly(HEMA-*g*-oligo(D)lactate), both dissolved in chloroform. Among other characteristics, the degradation of the obtained film was compared with the degradation of a film cast from a solution of a single enantiomer of the graft copolymer. Slower degradation was observed for the 1:1 blend of the L- and D-forms than for the single enantiomer. Stereocomplex formation from water was not investigated and is most likely not possible due to the high grafting density.

We realized the importance of avoiding organic solvents for the dissolution of the individual enantiomeric polymers to be used for the formation of stereocomplex hydrogels when aiming at biomedical applications such as the delivery of pharmaceutically active proteins. Therefore, we prepared biodegradable and biocompatible hydrogels based on dextran (a natural occurring polysaccharide) grafted with OLA and investigated the minimum and maximum length of the grafts required to form stereocomplexes after mixing and retaining water-solubility before mixing, respectively. The results and the application as a protein delivery device will be summarized in the next section.

Dextran-g-OLA Stereocomplex Hydrogels

In our Department we designed a hydrogel system based on dextran in which crosslinking is established by stereocomplex formation between lactic acid oligomers of opposite chirality. First, we investigated whether an 'operation window' of lactic acid chain lengths is present, in which stereocomplex crystallization would occur whithout homocrystallization of the individual enantiomers. Therefore, we isolated monodisperse lactic acid oligomers by preparative HPLC, from a polydisperse mixture obtained by conventional ring opening polymerization of L- or D-lactide. It was shown that crystallinity was present in individual D- or L-oligomers with a degree of polymerization (DP, *i.e.* the number of lactic acid repeating units) ≥ 11. On the other hand, in blends of D- and L-oligomers of lactic acid crystallinity (stereocomplexation) was already observed at a DP ≥ 7 (see Figure 1).(*37*)

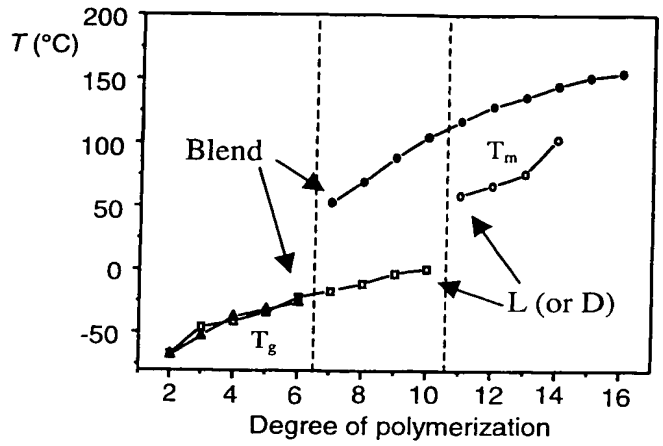

Figure 1. Glass transition (T_g) and melting temperatures (T_m) of lactic acid oligomers from differential scanning calorimetry measurements: OLLA (or ODLA) (open symbols) and 1:1 mixture of OLLA and ODLA (filled symbols). (Adapted with permission from reference 37. Copyright 1998 American Chemical Society.)

In the next step, polydisperse or monodisperse L- and D-lactic acid oligomers were coupled via their terminal hydroxyl group to dextran, yielding dextran-*g*-OLLA and dextran-*g*-ODLA, respectively, with variation in DP of the oligolactic acid and degree of substitution (DS, percentage of substituted dextran repeating units) (Figure 2). Interestingly, each product was soluble in water separately and upon mixing solutions containing OLLA- and ODLA-grafted dextran, hydrogels are formed at room temperature as demonstrated by rheological measurements.(*38*) As can be seen in Figure 3, the storage modulus of the obtained hydrogel strongly decreased upon heating to 80 °C, while it was restored upon cooling to 20 °C demonstrating the thermo-reversibility and the physical nature of the crosslinks. The storage modulus of the gels depends on the degree of polymerization of the lactate acid grafts and their degree of substitution on dextran. Mixtures of dextran-*g*-OLLA and dextran-*g*-ODLA containing monodisperse grafts with a DP lower than 11 did not result in a hydrogel. This is in contrast to the observation that stereocomplexation already can occur for non-grafted OLA chains with a DP $\geq$ 7. This difference can be explained by hampered stereocomplex formation once the oligomers are both coupled via their hydroxyl group to dextran (Figure 4A). Interestingly, gel formation was favored when one lactic acid oligomer was coupled via its

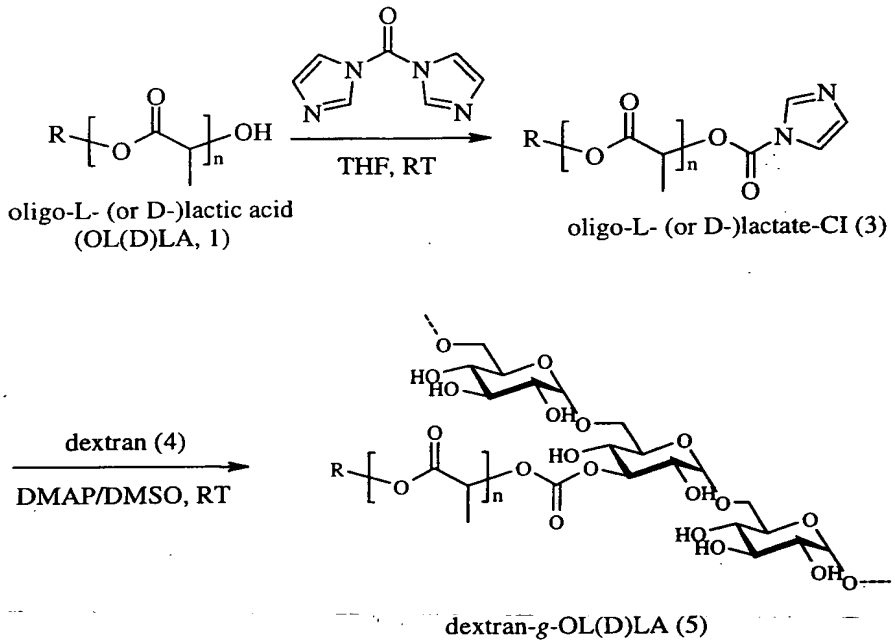

Figure 2. Synthesis of dextran-g-OLA. R represents a 2-(2-methoxy-ethoxy)ethyl (MEE) group.

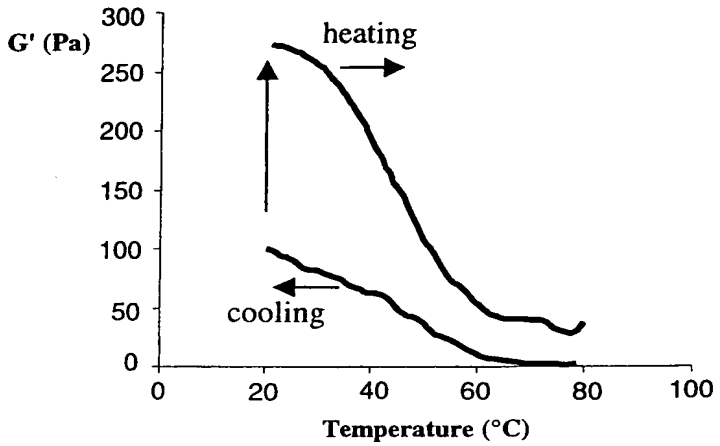

Figure 3. Storage modulus as a function of temperature of a dextran-g-OLA stereocomplex hydrogel ($DP_{average}$ = 9, DS = 3, 80 % water) upon heating and cooling. The vertical arrow reflects the increase in storage modulus in time at 20 °C to its original value. (Adapted from reference 38.)

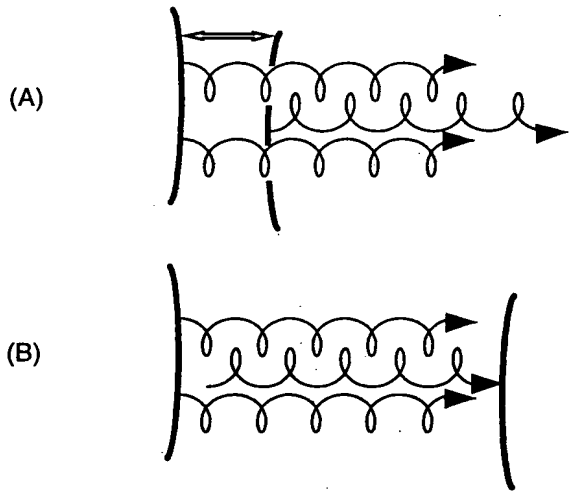

Figure 4. Schematic representation of stereocomplex formation in dextran-g-OLA hydrogels, showing the required unidirectional packing of the lactic acid chains in two cases: with both enantiomeric OLA chains connected to dextran via their OH terminus giving steric hindrance between the dextran chains (A) and one of either enantiomers coupled via the OH terminus and the other via its carboxylate terminus (B). The carboxylate termini are represented by the black arrowheads.

hydroxyl group whereas the oligomer of opposite chirality was coupled via its carboxylic acid group.(*39*) This is ascribed to the required parallel packing of the oligomers in stereocomplexes, *i.e.* in which all the chains are oriented in the same direction (as explained in Figure 4).(*24*)

Protein-loaded hydrogels were simply prepared by dissolving the protein in the dextran-*g*-OLLA/ODLA solutions prior to mixing. It was shown that under physiological conditions the gels are fully degradable.(*40*) The degradation time depended on the pH and the composition of the hydrogel, *i.e.* the number of lactate grafts, the length and polydispersity of the grafts and the initial water content, and varied from 1 to 7 days (Figure 5). Under non-degrading conditions (pH 4) the hydrogels, having a water content of almost 90% in their swollen states, appeared to be stable for more than 1 month. As shown in Figure 6, the gels showed a release of the entrapped model proteins (IgG and lysozyme) over 6 days and the kinetics depended on the gel characteristics, such as the polydispersity of the lactate grafts and the initial water content. The release of lysozyme was by diffusion, whereas for the bigger IgG, whose hydrodynamic radius approaches the estimated mesh size of the hydrogels, also swelling/degradation played a role in the release. Importantly, the proteins were quantitatively released from the gels and with full preservation of the enzymatic activity of lysozyme, emphasizing the protein-friendly preparation method of the protein-loaded stereocomplex hydrogel.

Conclusions

Hydrogels which are physically crosslinked by stereocomplex interactions have attracted recent attention for drug delivery purposes. Especially systems which are obtained from aqueous solutions of the two components are very attractive, since they provide a friendly environment for the encapsulation of highly sensitive bioactive substances (proteins, DNA, living cells). Moreover, it is anticipated that gel formation can take place *in situ* after injection of the low-viscous solutions. We have developed a versatile and fully degradable system obtained from water soluble dextran grafted with oligolactic acid chains. The mechanical properties, degradation profile and release of encapsulated compounds can be simply tailored by the composition of the materials. At present we are investigating means to extend the degradation time by changing the chemistry of the bonds between the grafts and the backbone. Also, the preparation of injectable microspheres is one of our goals. The biocompatibility of the system will be established, but no problems are expected in that respect since recent *in vivo* studies on chemically crosslinked dextran hydrogels already showed good biocompatibility.(*41*)

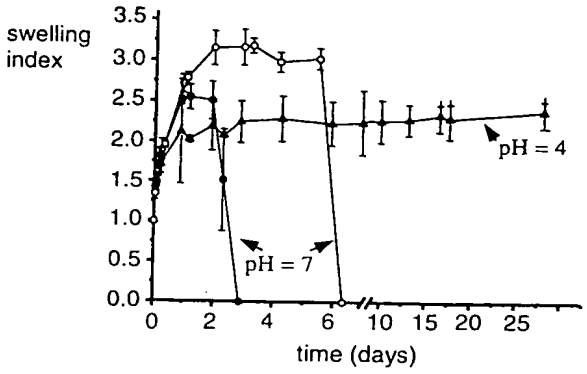

Figure 5. Swelling behavior of dextran-g-OLA stereocomplex hydrogels (DS = 6, 70 % water, 37 °C): high polydispersity lactic acid grafts ($DP_{average}$ = 12, $M_w/M_n \approx 1.25$, filled symbols) and low polydispersity grafts (DP = 11 to 14, M_w/M_n = 1.01, open circles). The filled triangles represent swelling under non-degrading conditions (pH 4) (average ± s.d., n = 3 or 4). (Adapted with permission from reference 40. Copyright 2001 Elsevier Science B.V.)

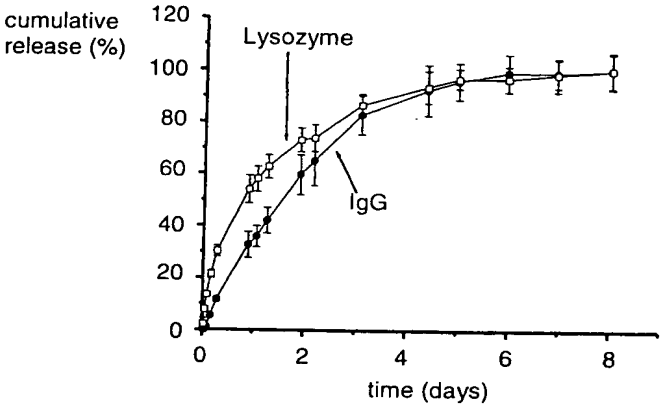

Figure 6. Release profiles of lysozyme (open squares) and IgG (filled circles) from dextran-g-OLA stereocomplex hydrogel with low polydispersity grafts (DS = 6, DP = 11 to 14, 70 % water, pH 7, 37 °C) (average ± s.d., n = 4). (Adapted with permission from reference 40. Copyright 2001 Elsevier Science B.V.)

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